EMBRYOLOGY OF MYOSURUS MINIMUS

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THERE is a close superficial resemblance existing between Myosurus and some of the lower forms such as the pines and the Selaginellaceae. For this reason the development of Myosurus presented itself as an inviting field of research. To be sure Bessey (1898) has already given the stages in the development of the pistils. My work in so far as it repeats these early stages serves as a confirmation of his description.

The developmental stages correspond very closely to those of Ranunculus, which has been investigated by Coulter (1898). The arrangement of the pistils, however, differs from that of Ranunculus in the fact that in Myosurus the receptacle is drawn out into a long central axis around which the pistils are arranged in spiral rows. Acropetal development of the pistils is very marked, so that the axis may lengthen nearly two inches after the pistils at the base have reached maturity (Pl. I, Fig. 8). The pistils formed late in the season around the apex of the axis are not pollinated. In respect to this conical arrangement of megasporophylls and the mode of development of the megasporangia there is at least a superficial resemblance to the pines and Selaginella.

As in most microsporophylls, so here there are four sporangia. At first the stamen is a mass of undifferentiated meristem (Pl. I, Fig. 1), but soon the limits of the sporangia become visible. The meristem cells within the areas which are to become sporangia begin to degenerate, while the first hypodermal layer becomes active in division. The cells, archesporial in character, divide periclinally to produce an outer primary wall cell and an inner primary tapetal cell. The former cell divides once

or twice, producing three or four layers in the wall. The latter cuts off spore mother cells which take the place of the degenerating meristem. The tapetal layer appears to be the outer layer of spore mother cells which have sacrificed their reproductive function for the nourishment of the other cells (Pl. I, Fig. 2). In this earliest stage of tapetal differentiation, some of the tapetal cells are binucleate. A little later practically all of them become binucleate and remain so till degeneration is accomplished (Pl. I, Fig. 3). This fact, to my mind, supports the theory that tapetal cells are potentially spore mother cells. The hereditary stimulus toward further division to form microspores is strong enough in them to carry the nucleus through one division, at which point their new function usurps control and arrests further development.

The mother spores are few in number and as the anther enlarges become rounded off and separated from each other (Pl. I, Fig. 3). The usual course of development now follows. The mother cells divide simultaneously in a given sporangium to form first two and then four cells arranged either tetrahedrally or in the same plane (Pl. I, Fig. 4). The last three stages in the maturity of the microspores are shown in Figs. 5, 6 and 7. There are developed on the wall three thin spots for the extrusion of the tube. The tube nucleus, the round one, and the generative nucleus, the oval one, are always present before the pollen leaves the anther. Upon germination the generative nucleus evidently divides, for two male nuclei are found in the pollen tubes. Degeneration of the tapetum is not very marked till the pollen grains are completely formed. In the mature anther not only the tapetum but all the other cells of the wall except the epidermis and first hypodermal layer have disappeared. This hypodermal layer develops heavy bands in the cell walls for breaking the anther.

The pistil appears in its earliest stage as a small swelling on the side of the receptacle (Pl. I, Figs. 8–9).

Soon this mass of cells bends a trifle downwards, and the anlage of the sporangium appears as a swelling in the distal axil formed by the juncture of the sporophyll with the receptacle (Pl. I, Figs. 8 and 10). The end of the sporophyll now begins to bend upwards and the ovule downwards. About this time the archesporial cell appears as a single large hypodermal cell (Pl. I, Fig. 13). When the ovule has bent downwards about 90° and the seed coats have begun to appear at its sides as slight elevations, the archesporium divides rapidly to form four megaspores, arranged approximately in a longitudinal row. The first two, however, often lie more or less side by side (Pl. I, Fig. 11). In a few cases I found only three megaspores. This may have been because of tardiness in the division of one of the cells. Meanwhile a groove has been forming in the upper surface of the carpel, and as the carpel continues to bend upwards the ovule is entirely enclosed by the sides and bottom of the groove. By the time the megaspores are formed, the upper convex surface of the ovule has fused with that portion of the pistil next to it. The ovule now hangs loosely downwards in the cavity thus formed. The nucellus continues its circular movement and by the time it has bent 180° the megaspore next to the chalaza, the functional one, has enlarged, while the others have begun to degenerate. When the ovule has described an arc of 225° the functional megaspore has made its first division. Between the two nuclei a large vacuole, which continues to enlarge with the growing sac, is formed (Pl. I, Fig. 12). The cells at the sides of the sac crowd in between the sac and the epidermis. At this stage the seed coats are prominent and have three or four layers of cells. In the next stage the nucellus has advanced about 90° farther in its cycle and each nucleus of the sac has divided. With the increase in size of the sac, the cells round about it begin to degenerate. The seed coats are now as long as the nucellus (Pl. II, Fig. 14). While the nucellus is passing through the next 45° of arc all the nuclei of the

sac divide, forming eight nuclei, four at each end of the sac. The nucellus now lies parallel to the axis of the receptacle with the micropyle, which is now completely formed, directed upwards (Pl. II, Fig. 15). One nucleus from each end of the sac moves toward the center where they fuse to form the definitive endosperm nucleus (Pl. II, Fig. 17). At first this nucleus is characterized by two nucleoli (Pl. II, Fig. 19), the others having only one. Maturation is now complete, and the cell which is to become the functional egg cell may lie between or at either side of the synergids.

The pistil has now reached its final stage. It makes a slight angle with the receptacle (Pl. II, Fig. 15). The stigmatic surface is marked by long spreading cells (Pl. II, Fig. 16). The presence of chloroplasts in the cells of the style and epidermis will be noted. The ovarian cavity is almost entirely filled with the ovule. It is lined with a layer of cells which represent the original epidermal layer before enclosure took place. In surface view they are seen to be irregular cells with wavy walls (Pl. II, Fig. 24). Immediately underneath this layer is one, or in some places two, layers of long interlacing cells formed from the fibro-vascular bundle. They form a firm basket-like structure.

Already, even before the definitive endosperm nucleus is formed, the pollen tubes are found in the micropyle. About the time of the formation of the endosperm nucleus the pollen tube enters the sac (Pl. II, Figs. 18–19). The synergids at once begin to degenerate. As the tube passes into the sac the egg nucleus and the endosperm nucleus move towards each other (Pl. II, Fig. 19). The cytoplasm around the egg nucleus becomes denser and soon distinct from the other cytoplasm. It is not certain about the conduct of the generative nuclei, actual fusion stages not having been observed. Except for a few isolated observations the evidence points to the fact that the endosperm nucleus is fertilized by one of the generative nuclei and the egg nucleus by the other. The isolated

observations just mentioned were to the effect that both male nuclei were present in the sac after the endosperm nucleus had proceeded to divide. At any rate, whether the endosperm nucleus requires fertilization or not, it precedes the egg nucleus in division. This fact supports the theory that the endosperm is a portion of the gametophyte.

The first division of the egg cell is periclinal, forming two cells, the distal one of which is destined to give rise to the embryo and the other the suspensor. These two cells then divide almost simultaneously, the former transversely, lengthening the suspensor, the latter longitudinally. Either may, however, precede the other in dividing. The four-celled stage is formed by a division of the two cells in a longitudinal plane at right angles to the first plane. One or both, generally one, of the cells of the suspensor divides transversely, thus completing the suspensor which possesses three or four cells (Pl. II, Fig. 20). The proximal cell often broadens at its base. The next division of the embryo cells is at right angles to the other two planes of division and results in an eight-celled stage (Fig. 20). About this time the distal cell of the suspensor makes two longitudinal divisions at right angles to each other (Pl. II, Figs. 21–22). Numerous divisions follow in the usual manner (Figs. 21–22). In the last stages the exact arrangement of cells was hard to determine on account of the difficulty in sectioning the hardened wall of the sac. But the shape of the mature embryo with its two cotyledons and the approximate arrangement of cells are shown in Fig. 23. The embryo lies in a cavity about twice its size and so does not come into contact with the endosperm except at the base of the suspensor.

The development of the endosperm is essentially similar to that of Ranunculus. The endosperm nucleus, fertilized or not, divides successively to form several nuclei distributed throughout the cytoplasm which radiates from them as centers. The antipodal cells number-

ing three, except in one case where four were present, become distinctly separated from the rest of the cytoplasm of the sac. After the first division of the egg cell the endosperm nuclei with their cytoplasm move to the periphery of the sac, where they form a complete lining one cell in thickness. The central portion of the sac is filled with a large vacuole (Pl. II, Fig. 20). The nuclei continue their divisions till the whole sac is filled with endosperm. This occurs about the eight-celled stage of the embryo.

There is considerable variability in the time when the walls appear, but it can be safely stated that they generally appear a little after the eight-celled stage of the embryo (Pl. II, Fig. 22). However, they may occur when the sac is lined with the single layer of nuclei. The antipodals can still be seen, but are on the road to degeneration. In one case I found a peculiar condition of the endosperm. At one end of the sac were the antipodals and at the other the synergids and egg-cell. Scattered in the cytoplasm of the central portion were three large, oval, endosperm nuclei containing several large nucleoli. One of them was dividing amitotically.

The endosperm nuclei, like the pollen mother cells, always divide simultaneously. There is a rhythmical succession of activities. This rhythm is so closely followed that every nucleus is found in the same phase of mitosis at the same time. This condition is localized in individual embryo sacs and there must be, therefore, a mutual reflex stimulus among the nuclei of a given sac.

As the embryo sac proceeds in its development the cells of the nucellus degenerate (Pl. II, Fig. 14). By the time fertilization takes place all have disappeared except the original epidermal covering of the nucellus (Pl. II, Fig. 18). At this stage the seed coats which possess three or four layers of cells have grown considerably beyond the nucellus to form the micropyle. When the endosperm nuclei take a peripheral position, the original epidermal layer begins to degenerate, except a few cells

at the base of the suspensor (Pl. II, Fig. 20). The walls of the cells composing the innermost layer of the seed coats become somewhat thickened and rounded. Later the cells increase greatly in size and become very stony in texture. All the other layers except the external one, which forms a delicate membrane, disappear.

By some botanists Myosurus has been considered a low type from which the monocotyledons and dicotyledons took their origin. This view was based principally upon its external vegetative structure. If it stands on the border line between these two groups, one would expect to find in its embryology features common to both and perhaps one well-developed cotyledon and another rudimentary one. But this is not the case. The cotyledons from their first appearance clear through the period of germination are exactly equal. And, furthermore, the other features of development are typically dicotyledonous, corresponding to those of the other Ranunculaceæ. It is, however, to be regarded from its embryological and vegetative fatures as one of the lowest, if not the very lowest, of the dicotyledons.

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LITERATURE CITED.

1898. Bessey, E. A. The Comparative Morphology of the Pistils of the Ranunculaceæ, Alismaceæ and Rosaceæ. *Bot. Gaz.*, XXVI, 297–313.

1898. Coulter, J. M. Contribution to the Life History of Ranunculus. *Bot. Gaz.*, XXV, 73–88.

EXPLANATION OF PLATES

Drawings were made with camera lucida. For clearness, all figures of pistils or their parts are placed on the page in the same relation to the drawing of the flower (Fig. 8) as exists in nature between the flower and the parts here represented.

PLATE I

FIG. 1. Cross section of an anther showing the region in which two of the sporangia develop. The cells are now undifferentiated. ×590.

FIG. 2. Cross section of anther showing a single microsporangium. Four mother cells surrounded by the tapetal layer. The cells of the wall are arranged radially. × 590.

" 3. Longitudinal section of anther showing the epidermis, two hypodermal layers of the wall, the tapetum composed of binucleate cells, and two mother spores. × 590.

" 4. Four tetrahedrally arranged microspores within the wall of the spore mother cell. × 590.

" 5. Three microspores just after the dissolution of the mother wall. × 590.

" 6. Still later stage of microspore with a thick wall. The nucleus has not yet divided. × 590.

" 7. Mature microspore showing the large round tube nucleus and a generative nucleus. The three thin spots in the wall are visible. × 590.

" 8. Longitudinal section through a young flower. *A*, anther. *B*, petal. *R*, receptacle. *S*, sepal. *P*, pistil. *N*, nucellus. × 35.

" 9. More highly magnified anlage of a pistil. × 365.

" 10. A little later stage showing the anlage of the ovule. × 365.

" 11. The nucellus has bent downwards 90°. The seed coat is appearing at the left, and four megaspores are present. × 365.

" 12. Two-celled stage of the embryo sac. Seed coats appear at the sides. × 365.

" 13. First appearance of archesporium. The pistil has bent upwards to enclose the nucellus. × 210.

PLATE II

FIG. 14. Four-celled stage of the embryo sac. The seed coats have grown to the end of the nucellus. The cells about the sac are degenerating. × 365.

" 15. Mature pistil and embryo sac with its three antipodals, two synergids, egg nucleus and endosperm nucleus. The dotted lines show the position of the fibero-vascular bundles. × 45.

" 16. The stigma of Fig. 15 highly magnified. × 210.

" 17. Embryo sac showing the three antipodals, the two synergids and egg nucleus, and in the center the other two nuclei fusing. × 365.

" 18. The pollen tube entering the embryo sac. The tube nucleus and two generative nuclei are present. The definitive endosperm nucleus is not yet formed. The synergids are degenerating. × 340.

" 19. Pollen tube within embryo sac. Its end, containing two generative nuclei and tube nucleus, is between the large endosperm and the smaller egg nucleus. The degenerating synergids are shown at the side. × 590.

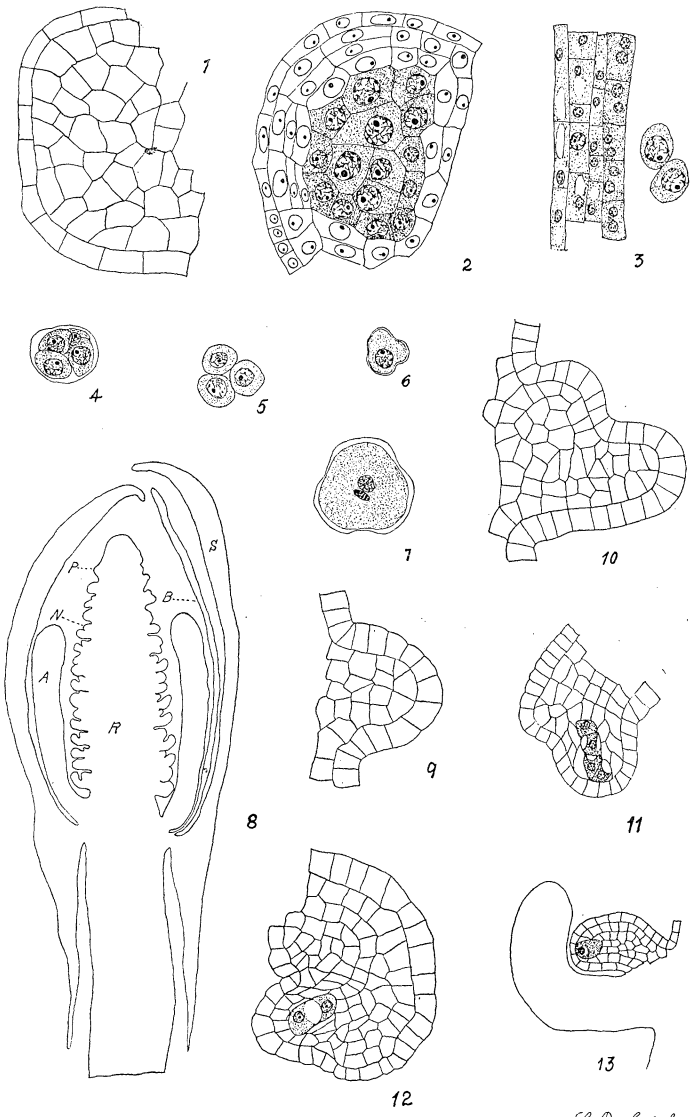
" 20. Eight-celled stage of the embryo. Three cells present in the suspensor. The endosperm nuclei are in the periphery of the sac. Three scattered nuclei of the degenerated nucellus still apparent, only five cells of the epidermal covering being left as a support for the suspensor. × 340.

" 21. A little later stage of the embryo. The distal cell of the suspensor has divided to form four cells, only two of which could be shown. × 340.

" 22. Still larger embryo. In this case there were four cells in the suspensor before the distal one divided to form four. At the left the endosperm with its cell walls is shown. × 340.

" 23. Mature embryo showing two cotyledons and three cells in the suspensor. × 365.

" 24. Surface view of epidermal cells lining the loculus. × 365.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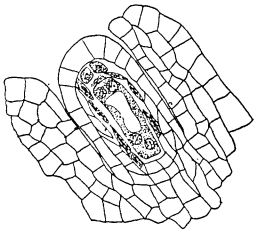

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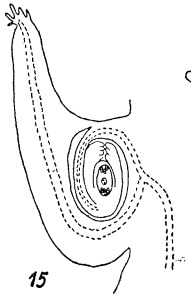

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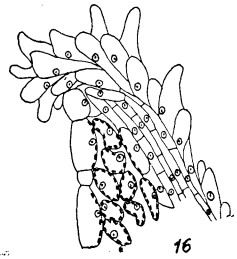

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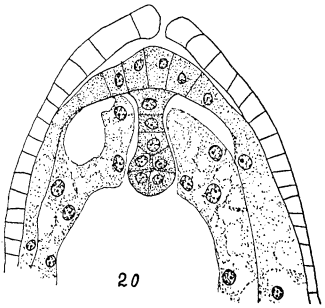

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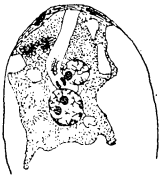

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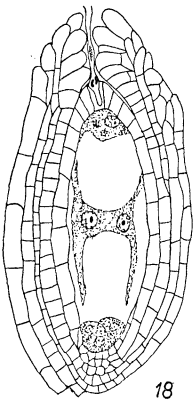

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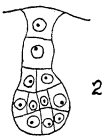

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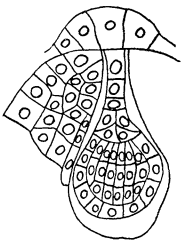

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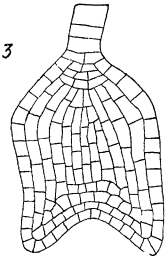

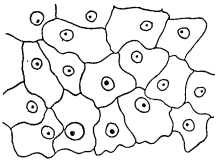

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